Joyce v. Natzmer
Hall, Vande Sande & Pequignot, LLP
4615 North Park Avenue
Suite 919
Chevy Chase, MD 20815
Tel.: (301) 657-1282
Fax.: (301) 657-1283

RECEIVED
CENTRAL FAX CENTER

JAN 11 2006

FACSIMILE COVER SHEET

To:	United States Patent and Trademark Office
Fax:	(571) 273-8300
Art Unit:	**1645**
Att:	**Special Program Examiner; TC 1600**
From:	Joyce von Natzmer
Appl. No:	**10/823,784 - PETITION TO MAKE SPECIAL**

Date: January 11, 2006

Pages (including this cover sheet): 7

Attached hereto is/are the following for the subject application:

- **Petition to Make Special** (3 pgs.);
- Application Notes, Nature Methods: i-ii, October 2005 (2 pgs.); and
- Form PTO-2038 to cover fee under 37 CFR §1.17(h).

Joyce von Natzmer
Attorney for Applicants
Registration No. 48,120
Telephone: (301) 657-1282

I hereby certify that, on the date shown below, this correspondence is being facsimile transmitted to the Patent and Trademark Office, (571) 273-8300.

January 11, 2006

Joyce von Natzmer
Registration No. 48,120

RECEIVED
CENTRAL FAX CENTER

JAN 11 2006

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)
UHLMANN et al.	) Atty. Dkt. **3035-101**
Serial No.: 10/823,784	) Examiner: n/a
Filed: April 14, 2004	) Group Art Unit: 1645

For: **METHOD OF DETECTING EPIGENETIC BIOMARKERS BY QUANTITATIVE METHYLSNP ANALYSIS**

PETITION TO MAKE SPECIAL UNDER 37 CFR § 1.102

Att. Special Program Examiner of TC 1600
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Applicants submit herewith their petition to accord special status to the subject application on the grounds that the invention contributes to the diagnosis and/or prevention of cancer (MPEP §708.02 (X)). The fee under 37 CFR §1.17(h) is submitted herewith.

01/12/2006 TL0111 00000008 10823784
01 FC:1464 130.00 OP

STATEMENT EXPLAINING THE PRESENT INVENTION'S CONTRIBUTION TO THE DIAGNOSIS/PREVENTION OF CANCER

General

Methylation of nucleotides is a key element of epigenetic control of genomic information in mammals. As explained in the background section of the disclosure, aberrant DNA methylation is often associated with tumorgenesis. The invention is directed at the detection of the methylation status of nucleotides, such as CpG dinucleotides, and the diagnosis of cancer or a predisposition therefore via such detection. In many embodiments, the method is highly accurate, rapid, quantitative and/or when, e.g., the method is performed with samples derived from certain body fluids (serum, urine etc.), non-invasive. The methods of the present invention take advantage of the fact that certain agents, such as bisulfites, may create single nucleotide polymorphisms (SNPs) in a nucleic acid molecule, which allows, after amplification and sequencing of the amplification product, determination of whether or not a methylation existed at a predetermined position of the original nucleic acid molecule. This methylation in turn may be indicative of cancer or a predisposition therefore. An "application note" that was published in the *October 2005 edition* of *"Nature Method,"* which is instructive in the context of the present invention, is enclosed.

The claims

The claims are directed at detecting the methylation status of nucleotides and using such detection, e.g., for the diagnosis of cancer. Even where the claims do not directly refer to the diagnosis of cancer, they mostly cover methods that allow such a diagnosis. Claims that specifically refer to the diagnosis of cancer are, and were at the time of filing, part of the application.

Claim 12, as originally filed is directed at a method for the diagnosis of a pathological condition comprising detection of the methylation status of a nucleotide at a

predetermined position in a nucleic acid molecule. The methylation status of the nucleotide is indicative of a pathological condition. This pathological condition is identified in claim 13 as, among others, cancer. In claim 14, the cancer is said to be a primary tumor, a metastasis or a residual tumor. Finally, claims 15 and 16, specify the primary tumor to be glioma (claim 15), in particular an astrocytoma, oligodendroglioma, an oligoastrocytoma, a glioblastoma, a pilocytic astrocytoma (claim 16).

The fee under 37 CFR §1.17(h) is submitted herewith. However, the Commissioner is authorized to charge deposit account no. 50-3135 as required for consideration of this submission.

The Special Program's Examiner of TC 1600 reviewing this petition is urged to call the undersigned at the telephone number provided below for any questions that might arise during the consideration of this petition.

Respectfully submitted,

By _____

Joyce von Natzmer
Attorney for Applicants
Registration No. 48,120
Hall, Vande Sande & Pequignot, LLP
10220 River Road, Suite 200
Potomac, MD 20815
Telephone direct: (301) 657-1282
Telephone main: (301) 983-2500

January 11, 2006

Enclosure(s)

Pyro Q-CpG™: quantitative analysis of methylation in multiple CpG sites by Pyrosequencing®

Pyro Q-CpG from Biotage gives a new dimension to DNA methylation studies by quantitatively measuring the individual degree of methylation of consecutive CpG sites consistently over time. This reveals previously unseen patterns of methylation.

Methylation of cytosines in CpG dinucleotides is an important regulator of gene expression in the human genome. Changes in methylation are now known to have a fundamental role in the development of a variety of tumors. Quantitative measurement of variation of methylation over time and among tissues will reveal important correlations with other biological phenomena.

Pyro Q-CpG is effective for the analysis of CpG methylation because it is capable of quantifying methylation in explicit sequence context, and is fast and easy to perform. Assay design is flexible, as the distance from the first base to be sequenced can be varied, and therefore, the primer can usually be positioned in a region free of CpG sites. In addition, there are four options for design: the assay can be performed in forward or reverse orientations and on either the top or the bottom strands.

The approach uses bisulfite treatment and PCR to differentiate methylated cytosine (^{m}C) from unmethylated cytosine (C), and Pyrosequencing to quantify the ratio ^{m}C/C at each site[1,2]. It was first reported in 2002 (ref. 3) and independently developed by other groups[4,5].

Quantification by Pyrosequencing

Pyrosequencing analyzes single-stranded DNA templates by synthesizing complementary strand. Briefly, the four nucleotides (A, T, G and C) are added sequentially by a Pyrosequencing instrument to DNA templates. For every successful nucleotide incorporation, pyrophosphate (PPi) is released. PPi is converted in enzyme-catalyzed reactions to drive light emission in a quantity that is proportional to the number of incorporations (Fig. 1). Therefore, peak heights in the Pyrogram™ inform on homopolymeric sequences and allele frequencies.

Principle of analysis

As with most methods for quantitative analysis of CpG methylation, CpG sites of genomic DNA are first chemically converted by bisulfite

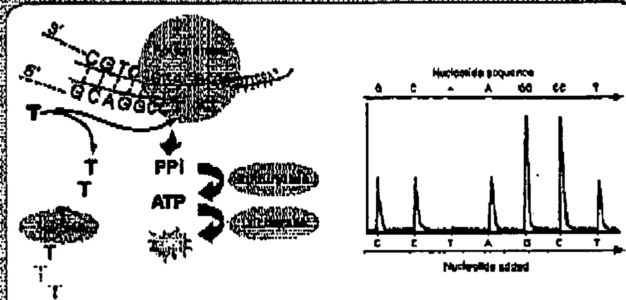

Figure 1 | The principle of Pyrosequencing and the output Pyrogram™. Double peak heights indicate incorporations of two nucleotides in a row.

treatment and then amplified by PCR. In this process, C is converted to uracil (U), whereas ^{m}C remains unchanged. In the subsequent PCR, U is amplified as thymine (T), and ^{m}C is amplified as C. In the Pyrogram, ^{m}C and C are therefore represented as C and T peaks, respectively. These peak heights are proportional to the number of methylated alleles at each CpG site (Fig. 2).

Experimental considerations

Sequence context is an important control because bisulfite-treated, PCR-amplified DNA is A-T rich, which decreases sequence variation. Pyro Q-CpG therefore guarantees that the correct sequence was analyzed.

Pyro Q-CpG assays can contain an internal control for bisulfite treatment. C that is not followed by G in sequence is not methylated, and should be fully converted to T by bisulfite and PCR. To confirm this, all templates should show only T and zero C in this position (Fig. 2).

Pyro Q-CpG is practical in terms of starting material and throughput. DNA is readily analyzed from both fresh frozen tissue as well as the short PCR fragments that are typical of paraffin-embedded tissue, in which restriction fragment analysis would be difficult. The analysis takes about one hour for 96 specimens in parallel, at a fraction of the cost and time of equivalent dideoxy sequencing reactions.

Robert England & Monica Pettersson

Biotage AB, Kungsgatan 76, 753 18 Uppsala, Sweden. Correspondence should be addressed to [illegible]@biotage.com.

PUBLISHED ONLINE 27 SEPTEMBER 2005; DOI:10.1038/NMETH800

APPLICATION NOTES

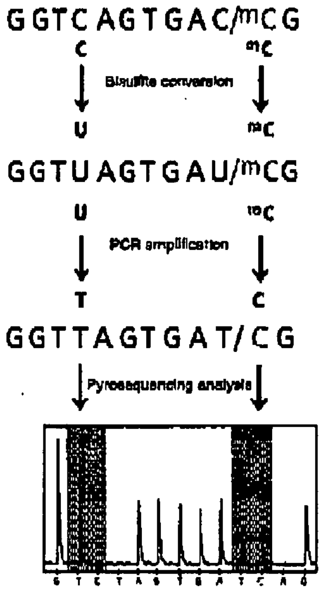

Figure 2 | Principle of analysis. Unmethylated C (red) and methylated C (green) are differentiated by bisulfite treatment and PCR. The ratio C/mC at each CpG site (peaks in orange column) is measured in sequence context. C not followed by G acts as control for the bisulfite step (blue column).

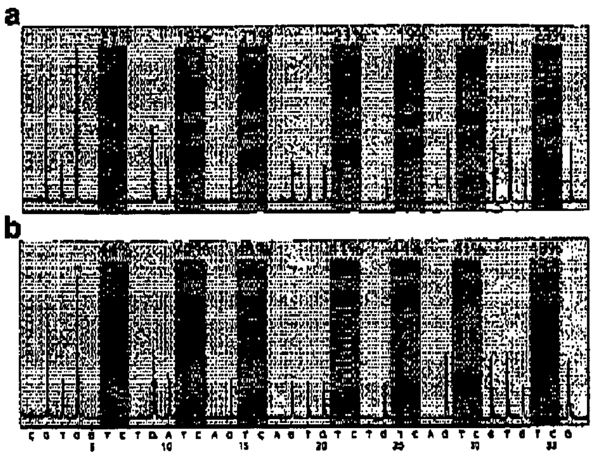

Figure 3 | Data output from analysis of seven CpG sites in the *p16^INK4a* promoter. Methylation levels in primary tumor (**a**) and metastatic lymphnodes (**b**) in head and neck cancer. Data courtesy of R Krahe, M.D. Anderson Cancer Center.

Workflow

Commercial reagent kits are available for bisulfite treatment of human genomic DNA. The PCR is performed with one of the PCR primers biotinylated. This is required for the conversion of the PCR product to single-stranded DNA templates. A sequencing primer is then added which anneals to the single-stranded DNA template. Analyses are automated, allowing routine processing of 96 samples in parallel.

Pyro Q-CpG

Pyro Q-CpG is a complete solution from Biotage for methylation analysis that exploits the advantages of Pyrosequencing technology. Pyro Q-CpG comprises PyroMark™ instrumentation and optimized software for CpG methylation analysis, as well as validated Research Use Only (RUO) tests for measuring methylation in specific genes. The PyroMark RUO tests include assays for methylation in *p16^INK4a*, *[illegible]*, *MLH1*, *MGMT*, *RASSF1A*, as well as Prader-Willi and Angelman syndromes, with more tests in the pipeline. Instrument users can access over [illegible] assays in the PyroMark online assay database, which contains assays for single nucleotide polymorphisms (SNPs), mutation and methylation analysis. For researchers who prefer to outsource methylation analysis, Biotage offers consultation for custom methylation assay design and laboratory services for sample analysis, including bisulfite treatment.

Summary

Pyro Q-CpG from Biotage offers several advantages for methylation analysis. Primarily, reproducible quantification of consecutive CpG sites is easy and fast on 96 samples in parallel. Assay design is flexible. The method is versatile for a range of analyses, from single and multiple consecutive CpGs to estimations of global methylation. As methylation of each site is measured in the context of the DNA sequence, software automatically performs quality control of the raw data to ensure that the expected sites were analyzed. Furthermore, Cs not followed by a G are used as quality control to evaluate whether the bisulfite treatment went to completion, thereby ensuring reliable data. The method is suitable for analysis of fresh, frozen, fixed and paraffin-embedded specimens.

Pyro Q-CpG is a valuable approach for acquiring quantitative methylation data that are comparable over time. Pyro Q-CpG can thus further our understanding on the variability of methylation with external variables such as treatment, individual and tissue sample (**Fig. 3**), which is a prerequisite for developing models describing methylation, gene expression and drug response.

1. Uhlmann, K., Brinckmann, A., Toliat, M.R., Ritter, H. & Nürnberg, P. Evaluation of a potential epigenetic biomarker by quantitative methyl-single nucleotide polymorphism analysis. *Electrophoresis* **23**, 4072–4079 (2002).
2. Colella, S., Shen, L., Baggerly, K.A., Issa, J.P. & Krahe, R. Sensitive and quantitative universal pyrosequencing methylation analysis of CpG sites. *Biotechniques* **35**, 146–150 (2003).
3. Tost, J., Dunker, J. & Gut, I.G. Analysis and quantification of multiple methylation variable positions in CpG islands by Pyrosequencing. *Biotechniques* **35**, 152–156 (2003).
4. Dupont, J.M., Tost, J., Jammes, H. & Gut, I.G. De novo quantitative bisulfite sequencing using the pyrosequencing technology. *Anal. Biochem.* **333**, 119–127 (2004).
5. Yang, A.S. *et al.* A simple method for estimating global DNA methylation using bisulfite PCR of repetitive DNA elements. *Nucleic Acids Res.* **32**, e38 (2004).
6. Ronaghi, M. Pyrosequencing sheds light on DNA sequencing. *Genome Res.* **11**, 3–11 (2001).